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BIOCHEMICAL GENETIC CHARACTERISTIC OF THE FARMED ATLANTIC SALMON (*Salmo salar*) STOCK DEVELOPED IN POLAND FOR THE RESTORATION PURPOSES

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ABSTRACT. Farmed stock of Atlantic salmon has been developed in Poland for the restoration purposes. Allozyme electrophoretic study has been carried out in order to evaluate the gene pool of the stock. Variation of genes coding for 13 enzymatic systems in the sample of 90 fish has been studied using horizontal starch gel electrophoresis. Among the 38 genetic loci scored, only four loci were polymorphic: *sAAT-3*^{*}, *IDDH-2*^{*}, *sIDHP-4*^{*}, and *mMEP-2*^{*}. Percentage of polymorphic loci was $P=10.5$, mean number of alleles per locus was 1.1, and average observed heterozygosity was $H=0.037$. As other studies on Atlantic salmon report more numerous polymorphic loci, it is possible that the hatchery operations caused limited erosions of genetic variability in the reared stock. This stresses the importance of using adequate numbers of parental fish in establishing the hatchery rearing operations, in order to minimize the founder effects and genetic drift.

Key words: ALLOZYMES, ELECTROPHORESIS, GENETIC VARIATION, HETEROZYGOSITY,
POLYMORPHISM, *Salmo salar*

INTRODUCTION

Natural reproduction of Atlantic salmon (*Salmo salar*) in Polish rivers is extremely limited (Wiktor 1989). In order to support salmon population, the breeding stock has been established under the fish farm conditions, securing annual production of the required amounts of smolts for stocking purposes (Bartel 1993). The breeding stock has been created thanks to the importation of eyed eggs of Atlantic salmon, caught on their natural spawning run in Daugawa River (Latvia). This river was chosen due to its close vicinity to Polish Baltic coast.

In this study, allozyme electrophoresis has been applied in order to estimate the state of the gene pool of the Atlantic salmon breeding stock, developed and perpetuated under the fish farm conditions.

MATERIAL AND METHODS

In 1995 two samples (45 fish each) of *Salmo salar* breeding stock offspring reared in AQUAMAR, Miastko, Poland, were obtained: one sample of two, and one of one year old fish. Whole fish were frozen and kept at -20°C until the electrophoresis.

Allele frequency data were derived from the electrophoretic separation of the enzyme products of 38 gene loci. Horizontal starch gel electrophoresis was performed on muscle, liver, and eye tissue according to the method of Vuorinen (1984). Three buffer systems were used: (A) a discontinuous lithium hydroxide-boric acid buffer, pH 8.1 (Ridgway *et al.* 1970), (B) an N- (3-aminopropyl – morpholine-citrate buffer, pH 6.5 (Clayton & Tretiak 1972), with pH changed as in Vuorinen (1984), and (S) a 0.135 M. tris-citrate buffer, pH 7.1 (Shaw & Prasad 1970). Histochemical staining followed Harris & Hopkinson (1976). Table 1 lists the genetic loci coding for the enzymes examined. The enzyme and locus nomenclature follows Shaklee *et al.* (1990). Genetic models follow Johnson (1984), Vuorinen (1984), and Vuorinen & Piironen (1984). Electrophoretic data were analysed using the computer program BIOSYS-1 of Swofford & Selander (1981), release 1.7.

RESULTS AND DISCUSSION

Among 38 gene loci studied, only four loci were polymorphic (Table 2). The observed level of heterozygosity and number of alleles per locus (Table 2) were lower than those reported in some studies (Verspoor 1988, Youngson *et al.* 1991), and similar to those reported in others (Vuorinen & Berg 1989). Some authors reported loci that were polymorphic in Atlantic salmon samples, and which were monomorphic in this study. For instance Johnson (1984) found polymorphism on *sMDH-A1*^{*} and *sMDH-B1*^{*}, Verspoor (1988) found polymorphic one of the *sAAT*^{*}, *CK-A*^{*}, *GPI*^{*}, *sMDH*^{*}, and *sMEP*^{*} loci, etc. Apparently, the examined stock of Atlantic salmon preserved substantial amount of its genetic variation, although the hatchery operations

TABLE 1

Proteins studied, their abbreviations and Enzyme Commission numbers. The tissues of expression are:

M = muscle, L = liver, E = eye. The buffer systems employed (A, B, S) are described in the Materials and Methods

Enzyme name	Enzyme number	Abbreviation	No. of screened loci	Tissue	Buffer
Aspartate aminotransferase	2.6.1.1	sAAT	2	M	B
		sAAT	1	L	B
Alcohol dehydrogenase	1.1.1.1	ADH	1	L	S
Creatine kinase	2.7.3.2	CK-A	2	M	A
		CK-B	1	E	A
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3PDH	2	M(L)	S
			1	L	S
Glucose-6-phosphate isomerase	5.3.1.9	GPI-A	1	M	A
		GPI-B	2	M	A
L-idoitol dehydrogenase	1.1.1.14	IDDH	2	L	A
Isocitrate dehydrogenase	1.1.1.42	mIDHP	2	M	S
		sIDHP	2	L	B
Lactate dehydrogenase	1.1.1.27	LDH-A	2	M	A
		LDH-B	2	E	A
		LDH-C	1	E	A
Malate dehydrogenase	1.1.1.37	mMDH	2	M	B
		sMDH-A	2	L	B
		sMDH-B	2	M	B
NADP ⁺ -dependent malic enzyme	1.1.1.40	mMEP	2	M	B
		sMEP	2	L(M)	B
Phosphogluconate dehydrogenase	1.1.1.44	PGDH	1	M(L)	B(S)
Phosphoglucomutase	5.4.2.2	PGM	2	M	S
Superoxide dismutase	1.15.1.1	sSOD	1	L	B(S)

possibly caused limited erosion of genetic variability in the reared stock. For comparison, the ancestral stock of Daugawa River should be examined.

The goal of the hatcheries designed to provide fish for a release into the wild should be to preserve, as nearly as possible, the genetic characteristics of the ancestral stock. In the hatchery environment, the rate of genetic changes in a stock can be greatly increased as a result of inbreeding or artificial selection (intentional or unintentional). An effective population number of a few hundred per generation is necessary to avoid long-term deleterious effects from inbreeding and genetic drift. Although ma-

TABLE 2

Allele frequencies, mean heterozygosity (H), and percentage of polymorphic loci (P, 99% criterion) in two samples of *Salmo salar*. H and P are based on 38 loci. For each sample 45 fish have been examined.

Following loci were monomorphic for *100 allele (unless other electrophoretic mobility stated):

*sAAT-1**, *sAAT-2**, *ADH*100*, *CK-A1**, *CK-A2**, *CK-B**, *G3PDH-2**, *G3PDH-3**, *GPI-A**, *GPI-B1**, *GPI-B2**, *IDDH-2**, *mIDHP-1**, *mIDHP-2**, *sIDHP-3**, *LDH-A1**, *LDH-A2**, *LDH-B1**, *LDH-B2**, *LDH-C**, *mmDH-1**, *mMDH-2**, *sMDH-A1**, *sMDH-A2**, *sMDH-B1**, *sMDH-B2**, *mMEP-1**, *sMEP-3**, *sMEP-4**, *PGDH**, *PGM-1*100*, *PGM-2*100*, *sSOD**

Locus	Allele	Sample		
		1-year old	2-year old	pooled 1- and 2-year old
<i>sAAT-3*</i>	<i>a</i> 100	0.745	0.859	0.820
	<i>b</i> 65	0.244	0.141	0.175
	<i>c</i> 120	0.011	0.000	0.005
<i>IDDH-1*</i>	<i>a</i> 100	0.386	0.443	0.418
	<i>b</i> -100	0.614	0.557	0.582
<i>sIDHP-4*</i>	<i>a</i> 100	1.000	0.967	0.983
	<i>b</i> 130	0.000	0.033	0.017
<i>mMEP-2*</i>	<i>a</i> 100	0.722	0.856	0.789
	<i>b</i> 160	0.278	0.144	0.211
H (%)		4.3	3.2	3.7
P (%)		7.9	10.5	10.5

ny fish farms regularly spawn several hundred (or more) adults per year, uneven sex ratios in the spawners, unequal fertilization rates of different individuals in mass spawning, and high variance in survival rates among families, may cause effective population number to be much smaller than the number actually spawned.

Loss of genetic material affects the immediate performance of the stock and might limit its flexibility to respond to changing environmental conditions in the future. A useful measure of the amount of genetic variability in a population is the percentage of the heterozygous individuals averaged over many gene loci (H). An important goal in managing salmon populations, however, is not to achieve a particular level of heterozygosity (H) that would show a wide range of values among salmon wild populations from different areas (Utter et al. 1989), and among populations within a drainage (Winans 1989). It is rather to ensure that the existing levels of genetic variability are not deteriorated by management practices.

One evaluation strategy is to monitor changes in heterozygosity levels over time in all hatchery stocks. However, because H is most strongly affected by changes in the

frequency of common alleles, serious losses of rare alleles can occur before significant decreases in heterozygosity levels are apparent. An early indication of small population size can be obtained by monitoring changes in allele frequency over time. The temporal method is best suited to the study of populations of small effective size, and may help to identify procedures that are successful in maintaining an adequate effective population size (Waples et al. 1990). Comprehensive yearly sampling program for the AQUAMAR hatchery stock could make it possible to monitor the nature and extent of genetic changes, and the results would provide some practices minimizing the effects of inbreeding.

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STRESZCZENIE

BIOCHEMICZNE CECHY GENETYCZNE STADA HODOWLANEGO ŁOSOSIA ATLANTYCKIEGO (*Salmo salar*) UTWORZONEGO CELEM RESTYTUCJI TEGO GATUNKU W POLSCE

W Polsce utworzono hodowlane stado łososia atlantyckiego w celu wykorzystania go do restytucji w Polsce. Przeprowadzono badanie elektroforetyczne allozymów zmierzające do oceny zasobów genetycznych tego pogłowia. Poziomą elektroforezę na żelu skrobiowym wykorzystano w analizie zmienności kodów genetycznych w 13 systemach enzymów w próbie obejmującej 90 osobników. Z przeanalizowanych 38 loci genetycznych tylko cztery okazały się polimorficzne: *sAAT-3*, *IDDH-2*, *sIDHP-4* oraz *mMEP-2*. Procent polimorficznych loci wynosił $P=10,5$, średnia liczba alleli w locus wynosiła 1,1, zaś średnia heterozygotyczność – $H=0,037$. Z uwagi na to, że w innych badaniach łososia stwierdzano większą liczbę loci polimorficznych postuluje się, że działania hodowlane mogły spowodować ograniczone uszkodzenie zmienności genetycznej. W pracy podkreślono znaczenie wykorzystywania w wylęgarniach odpowiednio dużej liczby ryb rodzicielskich, co zminimalizuje ujemne zjawiska genetyczne.

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